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THE DYNAMICS OF RAT ILEUM MUCOSA HEMOMICROCIRCULATORY STREAM PARAMETERS IN TRANSPLANTATION OF CRYOPRESERVED PLACENTA CONCOMITANT WITH ACUTE ASEPTIC PERITONEAL INFLAMMATION

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ABSTRACT

The experimental study has been carried out on the ileum extracted from 140 senior male rats. Histological and histochemical methods of study have been applied.

The response of all sections of hemomicrocirculatory stream has been detected in transplantations of cryopreserved placenta, expressed in the enlargement of its mean diameter with maximal parameters on day 5 of the experiment. Simulation of the acute aseptic peritoneal inflammation at first led to narrowing of arterioles by 5% and capillaries by 2.3% on day 2-3 of the experiment, followed by the enlargement of their diameters on day 14 of the experiment. During the experiment the venule section enlarged in the diameter on day 14 of the experiment. In transplantation of cryopreserved placenta concomitant with acute aseptic peritoneal inflammation the arterioles and capillaries at first significantly reduced in diameter by 4% and 2%, respectively, on day 2, and then enlarged in the diameter on day 3-10. During the experiment the venule section enlarged significantly in the diameter on day 14 of the experiment.

In administration of cryopreserved placenta concomitant with acute aseptic peritoneal inflammation the time period of its realization is shortened by 4-5 days.

Keywords: ileum, mucosa, hemomicrocirculatory stream, cryopreserved placenta, aseptic inflammation.

INTRODUCTION

The contemporary understanding of the hemomicrocirculatory stream (HMCS) encompass the system of complicated organization, providing with the regular flow of blood and tissue fluids, absorption and excretion of biochemical substrates, metabolites, bioactive substances. Among the interrelated and interdependent processes in the HMCS system the leading role is given to regularities of blood circulation in vessels with the diameter from 2 to 200 mcm, behavior of blood corpuscles, transcapillary exchange and ultrastructural peculiarities of microvessels [1, 2, 5, 6].

The last decade was marked by the novel approach to solving the problems related to the impairments of blood filling and blood outflow from the damaged organ in destructive lesions, induced by the inflammatory process in the body. Application of tissue transplantation and cryopreserved placenta preparations, in particular, as the method of correction is very effective [3, 4, 7], and the latter is the ground for carrying out the comprehensive study of the ileum mucosa HMCS's response to cryopreserved placenta transplantation.

PURPOSE

The research was aimed at the study of parameter dynamics of all sections of ileum mucosa HMCS in single time administration of cryopreserved placenta and administration of cryopreserved placenta concomitant with the experimental acute aseptic inflammation of rat peritoneum.

MATERIALS AND METHODS

The object of the experimental study was the ileum wall, extracted from 140 Wistar senior male rats. Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals" (2006, Annex 4) and Declaration of Helsinki on ethical principles for medical research involving human subjects.

The rodents have been assigned to four groups: Group I (n=5) included intact animals; Group II (n=45) included animals, who were single time subcutaneously administered with 0,125 $см^3$ fragment of placental tissue, sized 0,5 x 0,5 x 0,5 cm in conditions of small operational experimental biological clinic in compliance of all regulations of aseptic and antiseptics; Group III (n=45) was single time administered with intraperitoneal λ-carrageenan (Sigma - USA) (5 mg in 1ml saline per one animal) to induce the acute aseptic peritoneal inflammation; Group IV (n=45) was single time administered with subcutaneous cryopreserved placenta in conjunction with the simulated experimental acute aseptic peritoneal inflammation.

The animals were killed under thiopental anesthesia overdose in compliance with the scheduled time periods (1, 2, 3, 5, 7, 10, 14, 21, 30 days). Pieces of ileum were compressed by paraffin and epoxy according to conventional technique. The obtained blocks were sectioned and stained with Van Gieson hematoxylin-eosin, Hart's stain (paraffin sections), polychrome stain, methylene blue.

The inner diameter of HMCS (arterioles (Ar), capillaries (Ca), venules (Ve)) has been measured in the ileum mucosa. Microscope with Biorex 3 digital microphotohead (serial number 5604) has been used. Mathematical treatment of the material has been carried out using the conventional methods of variation statistics: calculation of mean values (M), error of mean (m), Student's T-test (t). Differences were considered to be reliable, when $p < 0.05$.

RESULTS AND DISCUSSION

The analysis of morphometric parameters of HMCS (Ar) resistive section showed the following changes in ileum mucosa in single time administration of cryopreserved placenta (Group II) during the experiment between the periods of investigation (Figure 1). In this way the (Ar) diameter enlarged

significantly on day 2, as compared with day 1 ($p<0,05$). Within the period of 3-5 days this parameter was increasing, but no significant difference was noted. On day 7 the parameter decreased a little, but while comparing it with the previous time period, no significant difference between them was observed. On day 10 the mean value of the (Ar) diameter significantly decreased, as compared with the previous time period. Within the 10-30 days their parameter was decreasing, but no reliable difference was observed.

While comparing the mean (Ar) diameter of Group II with the similar parameter of the intact group the increase of the parameter was observed, but the difference was significant during 2-7 days only.

The analysis of the parameter of mean (Ar) diameter in the group of rodents with induced acute aseptic peritoneal inflammation (Group III) has revealed the following changes between the time periods of the investigation. The significant decrease of the parameter was noted on day 2, as compared with day 1 ($p<0,05$). The value of this parameter increased a little on day 3 of the experiment; the reliability of difference was insignificant, as compared with day 2, whereas it was significant as compared with day 1, when $p<0,05$. The further analysis showed an abrupt increase of the parameter on day 5, and the reliability of difference was significant. Within the period of 5-14 days the parameter was increasing but no significant difference between the four time periods was noted ($p>0,05$). The decrease of the parameter was observed on day 21, and the difference between day 14 and day 21 was insignificant. On day 30 the parameter significantly decreased, as compared with day 21.

The comparative analysis of the mean (Ar) diameter between the group of rodents with induced acute aseptic inflammation and the group of intact animals showed a decrease of this parameter in Group III. If on day 1 the parameters were within the values of the intact group, then on day 2 and 3 a significant decrease of this parameter was noted ($p<0,05$). Within the period of 5-21 days the mean (Ar) diameter was significantly larger in Group III, as compared with the similar parameter of the intact group, with maximum value observed on day 14. On day 30 a significant difference of this parameter was detected between Group III and intact group of animals.

Morphometric analysis of the parameter of mean (Ar) diameter in the group of rodents, who underwent cryopreserved placenta transplantation in conjunction with acute aseptic peritoneal inflammation (Group IV) showed that during the experiment this parameter was changing between the time periods of the investigation (Figure 1). On day 2 a reliable decrease of the parameter was observed, as compared with day 1 ($p<0,05$). On day 3 it significantly increased as compared with previous time period. Within the period of 3-7 days the parameter was increasing, but the difference between these values was insignificant. On day 10 the parameter was tending to decrease, as compared with previous time period. On day 14 a reliable decrease of the (Ar) diameter value was detected, as compared with day 10. On day 21-30 the value of the parameter was within the values of the previous time period (when $p>0,05$).

The comparative analysis of the parameters of the mean (Ar) diameter between the intact group and Group IV showed significant decrease of the parameter on day 2 and reliable increase on day 3-10 with maximum value on day 5 in Group IV. On day 7-10 the parameter decreased a little, but the difference between the groups was reliable ($p<0,05$). While comparing the day 14, a significant decrease of this parameter was detected. On day 24-30 no significant differences in the mean values of the (Ar) diameter of the both groups was noted.

Therefore, the resistive section of HMCS of the ileum mucosa in transplantation of cryopreserved placenta was characterized by the gradual enlargement of the mean diameters with maximum value on day 5 and their recovery on day 10. In simulation of the acute aseptic peritoneal inflammation an abrupt narrowing of the diameters was noted on day 2-3 with subsequent enlargement during 5-14 days and incomplete recovery on day 30. Administration of cryopreserved placenta concomitant with acute aseptic

peritoneal inflammation showed at first an abrupt narrowing of (Ar) diameter on day 2 with subsequent enlargement on day 3-10 and total recovery on day 14.

The analysis of metric parameters of HMCS (Ca) metabolic section showed that in single time administration of cryopreserved placenta (Group II) the ileum mucosa changed differently during the experiment. Changes are presented in Fig. 2. It was established that the (Ca) diameters enlarged significantly on day 2, as compared with day 1 ($p<0,05$). Within the period of 3-5 days this parameter was increasing, but no significant difference between these time periods was noted. On day 7 the parameter decreased a little, as compared with the previous period, and no reliable difference between the time periods was detected ($p>0,05$). On day 10 the mean value of the (Ca) diameter decreased significantly, as compared with the previous time period ($p<0,05$). Within the 10-30 days this parameter was decreasing, but no reliability of difference was detected between them.

The comparison between the mean (Ca) diameter of Group II with the similar parameter of intact group showed a raise of the parameter, but the difference was significant for 2-7 days only (Fig. 2).

The analysis of the parameter of mean (Ca) diameter in the group of rodents with induced aseptic peritoneal inflammation (Group III) has revealed changes between the time periods of the experiment. A significant decrease of this parameter was detected on day 2, as compared with day 1 ($p<0,05$). On day 3 the value of this parameter increased a little and reliability of difference was not detected, as compared with day 2, but it was significant as compared with day 1 ($p<0,05$). Further analysis showed an abrupt raise of the parameter on day 5 and reliability of difference was significant. Within the period of 5-14 days the parameter was increasing and reliability of difference between these four time periods was detected. On day 21 a decrease of this parameter was detected and the difference between the day 14 and 21 was significant. On day 30 the parameter decreased a little and reliable difference was detected between them, as compared with day 21.

The comparative analysis of the mean (Ca) diameter between the intact group and Group III showed significant decrease of the parameter on day 2 and 3 (when $p<0,05$). Within 5-21 days the mean (Ca) diameter was reliably larger in Group III as compared with similar parameter of intact group, with maximum value on day 14. On day 21 it decreased, but was not similar to the values of intact group and was reliably large. On day 30 no significant difference was detected in the value of this parameter between Group III and intact group of animals.

Statistical analysis of the mean (Ca) diameter in the group of animals who underwent transplantation of cryopreserved placenta in conjunction with acute aseptic peritoneal inflammation showed changes in its values during the experiment (Fig. 2). A significant decrease of this parameter was detected on day 2, as compared with day 1 ($p<0,05$). On day 3 it significantly increased as compared with the previous time period. Within 3-7 days a raise of the parameter was noted but reliability of difference between the values was insignificant. On day 10 the parameter decreased a little, as compared with the previous time period. On day 14 a significant narrowing of the mean (Ca) diameter was detected. On day 21-30 the parameter value was within the values of the previous time period (when $p>0,05$).

The comparative analysis of the mean (Ca) diameter between Group IV and the intact group showed significant decrease of the parameter on day 2 and its significant increase on day 3-10 with maximum value on day 7 in Group IV. On day 24-30 no significant differences in the mean values of the (Ca) diameter of the both groups was noted.

Therefore, the metabolic section of HMCS of ileum mucosa responded differently in various types of the experiment. In this way during the transplantation of cryopreserved placenta a gradual increase of this parameter was detected with maximum value on day 5 and recovery on day 10. In simulation of the acute

aseptic peritoneal inflammation an abrupt narrowing of the diameters was detected on day 2-3 with their subsequent enlargement within 5-14 days and recovery on day 30. Administration of cryopreserved placenta concomitant with acute aseptic peritoneal inflammation reveals narrowing of the mean diameter of metabolic section of HMCS on day 2 with subsequent enlargement with maximum value of this parameter on day 7-10.

The statistical analysis of the mean diameter of the capacitive (Ve) section of HMCS of the ileum mucosa established that it changed dynamically, though differently. The results are presented in Figure 3.

In Group II the comparison of the parameters between time periods of the experiment showed the increase of the parameter on day 2 and 3; the difference between day 1 and 2 of the experiment was significant (when $p<0,05$). On day 5 the parameter value was maximal, but no significant changes between time periods of the experiment were detected as compared with day 3. On day 7 the parameter decreased, but no difference between the previous time period was noted. The period from 10 to 14 days is characterized by decrease of the parameter; if the comparison between these time periods is insignificant then the difference is reliable as compared with the previous time period (day 7). On day 21-30 the parameter was still decreasing, but the difference was also insignificant, as compared with the previous time period. The comparison between Group II and intact group of animals showed a significant difference in the parameters, measured on day 2-7 of the experiment only.

The analysis of the parameter of animals from the Group III, who underwent induced acute aseptic peritoneal inflammation, has revealed the following changes between the time periods of the experiment. On day 2 a reliable increased of the parameter was detected, as compared with day 1 ($p<0,05$). Within the 3-14 days the mean (Ve) diameter was enlarging, but no difference was detected between these time periods. On day 21-30 a significant narrowing of the diameters was detected, as compared with both the previous time period and between them.

The comparison of the intact group and Group III has revealed that significant difference between the parameters was detected within 2-21 days, with maximum value on day 14 of the experiment. The values, measured on day 1 and 30, were within the values of intact group.

Metric analysis of the parameters of animals from Group IV, who were administered with cryopreserved placenta in conjunction with acute aseptic peritoneal inflammation showed that the value of the mean venule diameter reliably increased on day 2, as compared with the value of day 1 and subsequent increase up to day 7, reaching the maximum value. The reliability of difference between days 2 and 10 of the observation was insignificant. Within 10-30 days this parameter was decreasing.

The comparative analysis of the mean (Ve) diameter between the animals from intact group and Group IV showed the reliable raise of the parameter within 2-10 days of the experiment, the reliability of difference accounted for ($p<0,05$); the reliability of difference was not noted during the other time periods.

Therefore, transplantation of cryopreserved placenta promotes the enlargement of diameters of capacitive section of HMCS with maximum value on day 5. In simulation of the acute aseptic peritoneal inflammation the enlargement of diameters is also evident with maximum value on day 14. Administration of cryopreserved placenta concomitant with acute aseptic peritoneal inflammation also promotes the enlargement of diameters of capacitive section with maximum value on day 7 of the experiment.

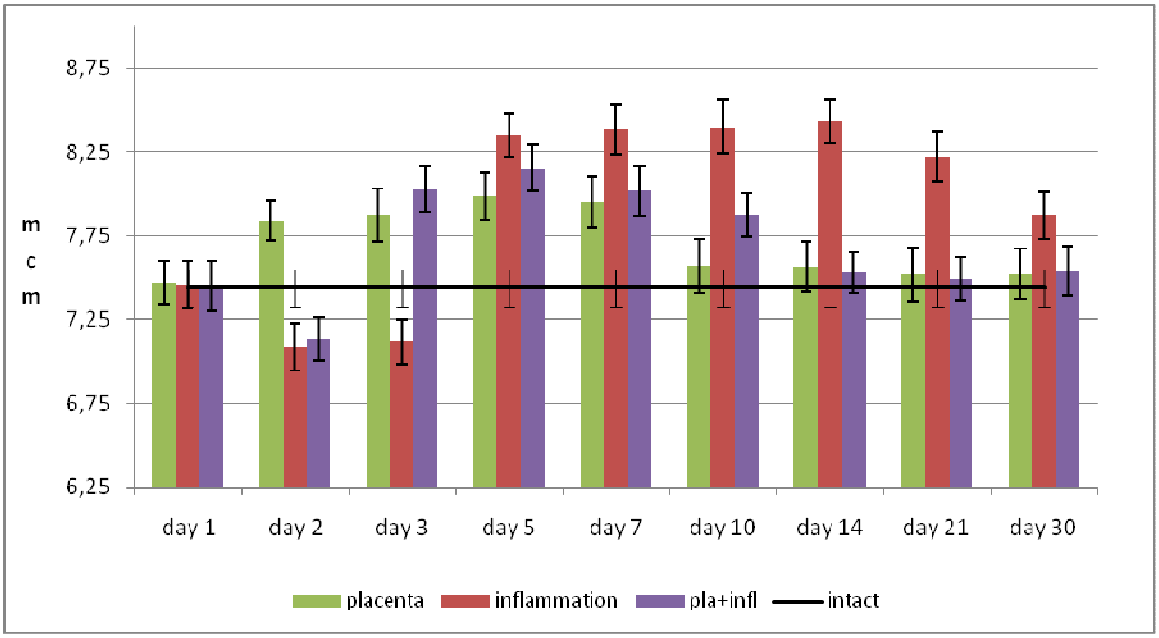

Figure 1. The comparative characteristic of the mean (Ar) diameter of the ileum mucosa

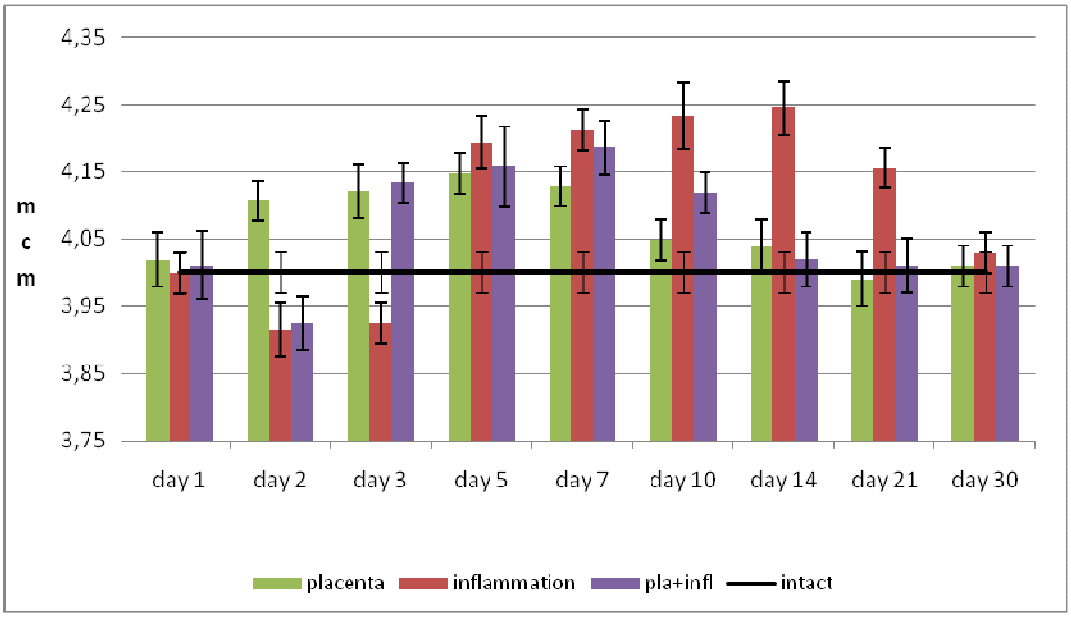

Figure 2. The comparative characteristic of the mean (Ca) diameter of the ileum mucosa

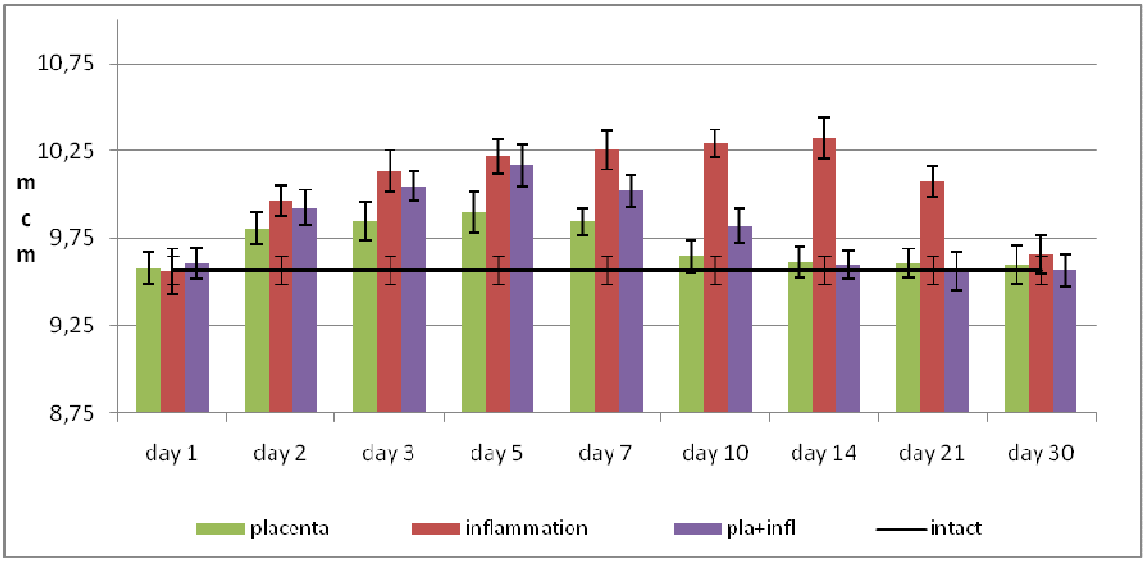

Figure 3. The comparative characteristic of the mean (Ve) diameter of the ileum mucosa

CONCLUSIONS

1. Transplantation of cryopreserved placenta evokes the response of all sections of hemomicrocirculatory stream due to the enlargement of its mean diameter with maximum values on day 5 of the experiment.

2. Simulation of the acute aseptic peritoneal inflammation at first led to narrowing of the arterioles by 5% and capillaries by 2.3% on day 2-3 of the experiment, followed by the enlargement of their diameters on day 14 of the experiment; the venule section enlarged in the diameter on day 14 of the experiment.

3. In transplantation of cryopreserved placenta concomitant with acute aseptic peritoneal inflammation the arterioles and capillaries at first narrowed in the diameter by 4% and 2%, respectively, on day 2, and then enlarged in the diameter on day 3-10 of the experiment; the venule section significantly enlarged in the diameter on day 14 of the experiment.

4. In administration of cryopreserved placenta concomitant with acute aseptic peritoneal inflammation the time period of its realization is shortened by 4-5 days.

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